SAMPLING TECHNIQUE FOR SOIL MACROARTHROPODS INHABITING FOREST FLOORS[1]

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ABSTRACT: Most soil fauna sampling techniques have been developed for microarthropods and are not suitable for sampling macroarthropods. This paper describes a better sampling methodology for the entire macroarthropods assemblage. A given sample area of 12.5cm by 25cm exempt from biases and obstacles is divided into three fractions (aerial, epigeic and endogeic). The collection of each fraction is adapted to the behavior of the soil fauna that it contains. Flying insects are first collected with a removable net attached to the top edge of the sampling mold, the litter is then gathered by hand, and finally the deeper organic layers are collected in a block. This method permits sampling of the soil by taking into consideration the vertical distribution of organisms. Samples stored as blocks of soil in polyethylene bags are sufficiently large and stable to insure the survival of organisms until extraction. This new methodology has certain advantages over traditional methods in that it allows a quantitative sampling of all soil organisms according to their vertical distribution.

Most sampling techniques for soil fauna have been developed by taking into consideration the microdistribution, small size and high density of microarthropods (especially Collembola and Acarina). These sampling parameters are not suitable for macroarthropods which are, in general, less numerous per unit area (Edwards 1967). The dimensions of the sample must therefore be proportional to the size of the organisms (Kaczmarek 1993), whereas the shape must attempt to maximize the representation of the soil under study. Indeed, the number of samples and the sample volume itself must attempt to compensate, through sufficient volume and number, the potentially contagious distribution of edaphic organisms (Górny and Grüm 1993, Huflejt and Karwowski 1993).

Good sampling must be representative of the environment under study (Kasprzak 1993) and avoid biases caused by nonhomogeneous features of the soil, which often harbor a particular fauna. Flogaïtis (1983) recommends that stumps, dead wood and proximity to trees should be avoided by keeping a constant minimal distance between these biases and the sample. Indeed, these distinctive features of the soil harbor their own characteristic fauna and are sources of contamination to be avoided during sampling. Stumps (Smith and Sears 1982), dead wood (Teskey 1976), animal excrement (Peck 1991), and fungi (Pielou and Verma 1968) are microhabitats to be avoided. Random sampling in an area exempt from biases and obstacles is thus recommended for the study of soil

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organisms. Górny and Grüm (1993) suggest a flexible rather than a rigid approach to sampling in respect of randomly chosen plots. Good judgement is therefore called upon when deciding whether to include or exclude habitat characteristics during sampling. For example, old cedar forest soils are associated with the presence of decomposing wood (Bergeron and Dubuc 1989). This distinctive feature should therefore be included in representative samples of such soils.

Geoffroy *et al.* (1981) and Zukowski (1993) mention that adult Diptera and Hymenoptera associated with the soil are rarely collected, even though these insects play an important role in the dynamics in soil assemblage at the larval stage or as parasitoids or parasites (Zukowski 1993). Traditional sampling techniques are not well suited for the characterization of highly mobile or flying macroarthropods associated with the soil.

We believe that the method described here is an improvement for the sampling and characterization of soil macroarthropods. It is the best possible compromise given the numerous constraints and variables associated with the collecting of soil organisms. The method has proven its superiority in an extensive forest soil ecology sampling program.

MATERIALS

The equipment includes: (1) An aluminum sampling mold 6mm thick and 20cm high, with a 12.5cm by 25cm sampling surface (Fig. 1). The mold's bottom edge is tapered to insure a good bite into the soil. (2) A removable Terylene net with an elastic band at its lower border (Fig. 1). This elastic border can be adjusted to the top edge of the sampling mold. It should be noted that the Terylene

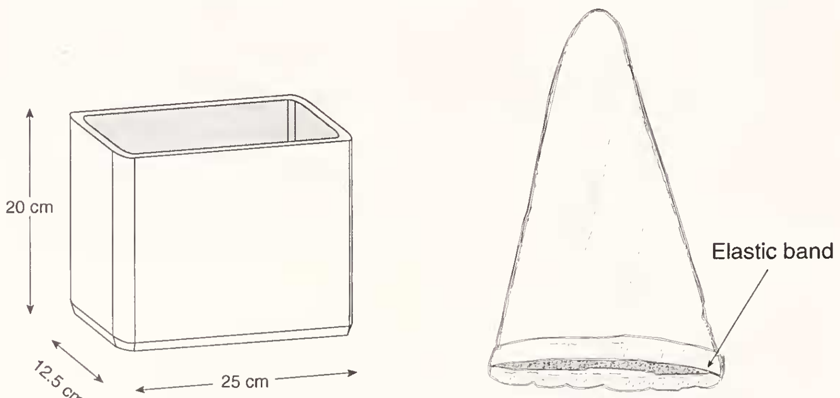

Figure 1. Sampling mold and removable net.

can be replaced by another material transparent enough to allow visual localization of the insects within the net. Terylene, however, is resistant to tearing and is not degraded by light, as is the case with other materials. (3) A wash flask filled with acetic alcohol. (4) Flexible tweezers. (5) Scintillation vials. Their advantage is that they are made of unbreakable plastic. One vial is required per soil sample. (6) A knife with a long blade. (7) A square shovel. (8) A 60cm by 90cm clean, white and smooth surface. A surface covered by melamine or Formica® is ideal. (9) Two-liter polyethylene bags. One bag per soil sample is required. (10) Ten-liter polyethylene bags. One bag per soil sample is required. (11) A measuring tape.

METHODOLOGY

This method was tested in boreal forests: including a deciduous forest (*Populus tremuloides* Michx.); a mixed forest (*Abies balsamea* (L.) Mill., *Picea glauca* (Moench) Voss and *Betula papyrifera* Marsh.); and a coniferous forest (*Thuya occidentalis* L. and *Abies balsamea* (L.) Mill.). These forests are situated in western Québec's clay belt, in the Duparquet Lake region (48°30' North, 79°15, West). See Bergeron *et al.* (1983) for a more detailed description of the vegetation and soils in this region.

Our technique is aimed at quantifying diversity and biomass at three macroarthropod groups: (1) flying adult insects associated with soils (Diptera and Hymenoptera), (2) fast-moving epigeic insects in the litter (larval and adult Staphylinidae, Carabidae, Arachnida, etc.) and (3) slower endogeic organisms found in the deeper organic fraction (Diptera larvae, etc.).

The collection of the three fractions (aerial, epigeic and endogeic) constituting a sample is carried out in eleven distinct steps: (1) Sampling plots are randomly chosen in areas exempt from biases and obstacles (as a function of included and excluded characteristics). Walking heavily is avoided since soil vibrations cause highly mobile insects to flee. (2) The sampling mold covered by the removable net is forcefully driven into the soil, so that the tapered edges of the mold penetrate the litter. (3) After one minute, the flying organisms can be found in the net. They rarely number more than one or two at a time. (4) A careful inspection allows one to locate and fix them using the wash flask filled with acetic alcohol. A single jet of alcohol through the net is enough to immobilize them. (5) After the insects are immobilized, the net is removed. Because of the surplus alcohol, the insects remain stuck on the inner surface of the net. With the flexible tweezers, the insects are transfered to the scintillation vials. This constitutes the aerial fraction of the sample. The vials must then be filled with acetic alcohol and labeled according to the sample to which they belong. (6) The litter (superficial layers composed of dead leaves, needles or twigs) contained within the mold is then delicately gathered by hand. This litter is transfered to two-liter polyethylene bags and constitutes the epigeic fraction of

the sample. During the first steps of the procedure, because the mold is driven into the soil, litter organisms are prevented from escaping. (7) The knife is used to cut through the soil along the inner edges of the sampling mold. The square shovel can be used to break roots that the knife cannot cut. (8) The mold is then removed, after which the block of soil is lifted from the ground with the square shovel and placed on the Formica® board. (9) The organic layers are then separated from the mineral layers so as to transfer in one block the organic layers to the ten-liter polyethylene bags. This fraction constitutes the endogeic fraction of the sample. In luvisols or podzols, the separation of organic layers from mineral layers is easily accomplished. (10) The bags containing litter, as well as the blocks of organic layers, are placed in an ice chest until they can be brought to the laboratory. Care must be taken so that the blocks are placed as they were originally found in the ground, with the top part of the blocks facing up, to avoid disturbing the organisms they contain. (11) The thickness of the litter and of the organic layers are measured in the hole left after the samples are taken. These data will later serve to calculate the volume of each fraction.

The total duration of the entire procedure in the field is ten minutes per sample. In the laboratory, the contents of the scintillation vials are transfered to vials that can better prevent the alcohol from evaporating. The fractions contained in the polyethylene bags are stored in a refrigerator at 4°C until extraction.

DISCUSSION

We believe our methodology possesses many advantages over previously used techniques. (1) Our approach for the choice of sample plots in areas exempt from biases complements that of Flogaïtis (1983). The elimination of biases associated with nonhomogeneous distinctive features of the soil insures a greater representativity of the sample. The greater volume collected, in comparison with traditional soil core techniques (Vannier and Vidal 1965), results in only slightly less flexibility regarding the choice of plots.

(2) Compared with the average small surface areas of samples generally used in studies of soil fauna (Murphy 1958a, 1958b, Vannier and Vidal 1965, Vannier 1966), that used in our method ($312.5cm^2$) is 12.5 times larger. Because of its small size, traditional coring, aimed especially at Acarina and Collembola, does not permit a true evaluation of larger (and hence less numerous per unit surface) macroarthropods (Edwards 1967). Vannier and Alpern (1968), however, underline the fact that a sampling surface should correspond to a precise surface area. That proposed in our method corresponds to $1/32\ m^2$. Vannier and Vidal (1965) recommend that the number rather than the size of samples be increased; it is preferable to have many smaller samples instead of one large sample. In that perspective, we are specifying that the sample size

must respect the physical characteristics such as the distribution, the size and the number per unit area of the organisms under study.

(3) The size of the sample proposed in our technique approaches that used by Vannier and Alpern (1968), who adopted a 20cm by 10cm surface, and that by Flogaïtis (1983) with 25cm^2. However, a rectangular rather than a square shape was adopted because, for a given surface, a rectangular shape samples on a longer transect, which better distributes the sampling effort and tends to reduce the influence of a contagious distribution.

(4) Our technique was tested in three types of forest soils. It is also suitable for agricultural soils, as well as many other types of ecosystems. With respect to its adaptability to different soil types, its versatility is comparable to the traditional coring sampling techniques.

(5) The volume of the samples and the methodology described herein allow the collection of every type of soil organism: flying organisms, organisms in the litter and those from deeper layers, be they macro or microarthropods. Our technique is nonselective, with each collected fraction adapted to the behavior of the organisms that comprise that fraction. Collecting in three fractions allows a rapid determination of the vertical distribution of organisms relative to a precise surface or volume, without risk of contamination, since our approach eliminates migration of organisms from one soil layer to another due to the layers' being collected separately. In general, traditional coring does not take the vertical distribution of organisms into consideration. Vannier and Alpern (1968) propose a method, inspired by Murphy (1958a), to study the vertical distribution of organisms, but the small sampling area (20 cm^2) of this technique is not suitable for macroarthropods. Our method, in this sense, complements that of Flogaïtis (1983) for the separation of sublayers of the sample. No other method allows the collecting of flying insects. With our proposed methodology, it is possible to sample adult Diptera and Hymenoptera. Although collected in lesser numbers than by the use of the terrestrial emergence cages (Martin 1977), these specimens can facilitate the identification of immature stages found in the soil, yield information on the phenology of the species involved and provide the first step in associating parasitoids and parasites with their hosts.

(6) Within a global approach to edaphic communities, our technique leads to a large quantity of microarthropods (Acarina and Collembola) because of their small size and the high densities they can reach (Edwards 1967). A subsampling of these groups, once the specimens are extracted, is therefore recommended (Niedbala and Rajski 1993).

(7) The quantitative approach of this technique allows the association of the organisms collected with a precise unit of surface or volume. The organisms collected in the epigeic fraction are the same as those collected by the European sifting technique described by Smetana (1971). The sifting technique is qualitatively more efficient due to the large volume of litter it allows one to process

The quantitative measure is however, more precise with a fixed soil surface such as proposed by our technique.

(8) Our collecting technique insures that the specimens are well preserved. The organisms in the first fraction are placed in alcohol while in the field and so remain in a perfect condition. Those found in the litter are subjected to only a delicate pressure during the manual collecting, which insures their good condition. The organisms contained in the third fraction sustain only a minimal pressure during the cutting out of the block of soil. The large size of the samples results in there being little disturbance for the organisms (Murphy 1958b, Vanier and Alpern 1968). Traditional coring, because of the small size of the samples it yields, exerts a physical pressure (Vannier and Alpern 1968) that can damage fragile specimens.

(9) The technique of collecting soil samples in the shape of blocks was initially proposed by Murphy (1958b) but the aim of his proposition was to later sample the blocks by careful coring without pressure. Our technique leads to larvae samples and requires more time for extraction than the smaller traditional samples. Edward and Fletcher (1970) and Leinnas (1978) studied the effects of storage on small samples. These were determined negligible for delays of 28 to 29 days. Rapoport and Oros (1969) reported variable effects for a delay of 120 days. They also showed that the use of polyethylene bags (rather than other materials) minimizes biases due to storage. Samples collected with our technique (in the shape of blocks and stored at low temperatures in polyethylene bags) allow an equivalent duration of storage, and probably a longer one because of the large volume involved. These blocks create a temporary environment sufficiently large and stable to insure the survival of the organisms they contain. A low refrigerator temperature (4°C) minimizes organism activity. Maturation is negligible and reproduction of most macroarthropods requires an adult sexual phase impossible under such conditions. The low levels of activity by individuals and the large sample volume minimize predation during storage.

The proposed technique is aimed mainly at forest soil macroarthropods but is equally suitable for all edaphic organisms. Our method allows the collection of individuals belonging to the aerial, epigeic and endogeic fractions of the soil. This quantitative method also considers the vertical distribution of organisms. The method is simple, inexpensive, efficient and rapid. It lends itself to many types of biological studies such as inventories, environmental impact evaluations, parasitism, voltinism, microhabitat studies and the vertical distribution of the soil fauna.

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